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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR §1.53 (b)(2).

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Attorney Docket No.: 5119-11200

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TITLE OF THE INVENTION
(280 characters max)

PIXEL ANALYSIS METHODS FOR THE RAPID SCREENING AND DETECTION OF MICROBES

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Washington, D.C. 20231

Derrick B. Brown

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒	Specification with Title Page	*Number of pages* 33	☒	Claims	*Number of pages* 5 (Claims 1-26)
☒	Drawings (figures 1-12)	*Number of pages* 14	☐	Abstract	*Number of pages*

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☒ A fee authorization is enclosed to cover the Provisional filing fees

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government>

☒ No

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

Signature _____ Date July 24, 2002

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☐ Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Filed: Herewith
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McDevitt et al.

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Respectfully submitted,



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PIXEL ANALYSIS METHODS FOR THE RAPID SCREENING AND DETECTION OF MICROBES

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BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method and device for the detection of analytes in a fluid. More particularly, the invention relates to the development of a sensor array system capable of discriminating mixtures of analytes, toxins, and/or bacteria in medical, food/beverage, and environmental solutions.

2. Brief Description of the Related Art

The development of smart sensors capable of discriminating different analytes, toxins, and bacteria has become increasingly important for clinical, environmental, health and safety, remote sensing, military, food/beverage and chemical processing applications. Many sensors capable of high sensitivity and high selectivity detection have been fashioned for single analyte detection. A smaller number of sensors been developed which display solution phase multi-analyte detection capabilities. One of the most commonly employed sensing techniques has exploited colloidal polymer microspheres for latex agglutination tests (LATs) in clinical analysis. Commercially available LATs for more than 60 analytes are used routinely for the detection of infectious diseases, illegal drugs, and early pregnancy tests. The vast majority of these types of sensors operate on the principle of agglutination of latex particles (polymer microspheres) which occurs when the antibody-derivatized microspheres become effectively "cross-linked" by a foreign antigen resulting in the attachment to, or the inability to pass through a filter. The dye-doped microspheres are then detected colorimetrically upon removal of the antigen carrying solution.

More recently, "taste chip" sensors have been employed that are capable of discriminating mixtures of analytes, toxins, and/or bacteria in medical, food/beverage, and environmental solutions. Certain sensors of this type are described in U.S. Application Ser. No. 10/072,800,

METHOD AND APPARATUS FOR THE CONFINEMENT OF MATERIALS IN A MICROMACHINED CHEMICAL SENSOR ARRAY, filed January 31, 2002 by McDevitt et al., which is incorporated by reference as if fully set forth herein. Disclosed therein are systems and methods for the analysis of a fluid containing one or more analytes. The taste chip array includes a sensor that has a plurality of chemically sensitive beads, formed in an ordered array, capable of simultaneously detecting many different kinds of analytes rapidly. An aspect of the system is that the array may be formed using a microfabrication process, thus allowing the system to be manufactured in an inexpensive manner.

Since concerns of bioterrorism attacks have become more pronounced, there has been increased interest in methods and systems for detecting microbes, particularly pathogens such as E. Coli O157:H7, B. anthracis/B. globigii, and Cryptosporidium, that may be used in chemical and biological attacks. As used herein, "microbe" refers to any microorganism, including but not limited to, a bacteria, spore, protozoan, yeast, virus, and algae. Numerous high quality tests exist for the detection of microbes within research laboratory settings. However, these tests are generally expensive, time consuming, and require substantial laboratory resources. For many real-world applications in the health and safety, environmental, military, treaty verification and homeland defense areas, it is desirable to monitor numerous locations simultaneously, even locations where the majority of the time there will be no dangerous levels of microbes present.

The "taste chip" and related technology described above may overcome many of the limitations discussed above with conventional tests for microbes. However, the beads that have typically been used in the taste chip systems are not optimized for the capture of larger analytes such as microbes.

It is therefore desirable that new methods and systems capable of discriminating microbes be developed for health and safety, environmental, homeland defense, military, medical/clinical diagnostic, food/beverage, and chemical processing applications. It is further desired that the methods and systems facilitate rapid screening of microbes to be used as a trigger for more specific and confirmatory testing. It is further desired that sensor arrays be developed that are

tailored specifically to serve as efficient microbe collection media.

SUMMARY OF THE INVENTION

Herein we describe systems and methods for the analysis of a fluid containing one or more analytes. The system may be used for either liquid or gaseous fluids. The system, in some embodiments, may generate patterns that are diagnostic for both individual analytes and mixtures of analytes. The system, in some embodiments, includes a plurality of chemically sensitive particles, formed in an ordered array, capable of simultaneously detecting many different kinds of analytes rapidly.

In an embodiment, a sensor array may contain one or more beads that contain macropores. Microbes such as bacteria, spores, and protozoa in a fluid may be captured in the macropores of the bead. In some embodiments, receptors, including, but not limited to, antibodies or semi-selective ligands such as lectins, may be coupled to a particle in an internal pore region of the bead to create a selective bead. In some embodiments, a visualization antibody may be introduced that may couple with the captured analyte to yield a colorimetric or fluorescence signature that can be recorded by the CCD detector. In some embodiments, a series of selective and semi-selective beads may be used in conjunction with the sensor array system described herein.

In some embodiments, a method for detecting microbes may include a multi-stage process wherein a fluid first undergoes a rapid screening and then, if warranted by the results of the screening stage, more specific and/or confirmatory testing. A sensor array including a macroporous bead may be used to conduct the specific and/or confirmatory testing.

Also described herein are methods for forming macroporous beads that may be used to detect a microbe. In an embodiment, a method for preparing a macroporous bead may include adding a dispersion of a hydrophilic emulsifier to an aqueous solution of a polymeric resin to form an oil-in-water emulsion, adding a solution of a hydrophobic emulsifier to the oil-in-water

emulsion to form a water-in-oil emulsion; then cooling the water-in-oil emulsion to form a polymeric matrix in which a plurality of oil droplets are dispersed. The oil droplets may be washed out of the pores of the polymeric matrix to form a macroporous bead.

BRIEF DESCRIPTION OF THE DRAWINGS

Features and advantages of the methods and apparatus of the present invention will be more fully appreciated by reference to the following detailed description of presently preferred but nonetheless illustrative embodiments in accordance with the present invention when taken in conjunction with the accompanying drawings in which:

FIG. 1 depicts a cross sectional view of a membrane based analyte detection device;

FIG. 2 depicts a cross sectional view of an alternate embodiment of a membrane based analyte detection device;

FIG. 3 depicts a cross sectional view of a membrane based analyte detection device that includes control valves in a sampling configuration;

FIG. 4 depicts a cross sectional view of a membrane based analyte detection device that includes control valves in a transverse washing configuration;

FIG. 5 depicts a schematic diagram of a membrane based analyte detection system in a collection configuration;

FIG. 6 depicts a schematic diagram of a membrane based analyte detection system in a flushing configuration;

FIG. 7 depicts a flow chart of a method of collecting samples;

FIGS. 8A-8E depict a method of analysis of particles captured by a membrane;

FIG. 9 depicts a schematic diagram of a membrane based analyte detection system that includes a sensor array detection device;

FIG. 10 depicts porous particles;

FIGS. 11A-D depicts a schematic diagram of a bead optimization method; and

FIG. 12 depicts a schematic diagram of a flow cytometer.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Herein we describe a system and method for the analysis of a fluid containing one or more analytes. The system may be used for either liquid or gaseous fluids. The system, in some embodiments, may generate patterns that are diagnostic for both the individual analytes and mixtures of the analytes. The system in some embodiments, is made of a plurality of chemically sensitive particles, formed in an ordered array, capable of simultaneously detecting many different kinds of analytes rapidly. An aspect of the system is that the array may be formed using a microfabrication process, thus allowing the system to be manufactured in an inexpensive manner.

In an embodiment of a system for detecting analytes, the system, in some embodiments, includes a light source, a sensor array, and a detector. The sensor array, in some embodiments, is formed of a supporting member which is configured to hold a variety of chemically sensitive particles (herein referred to as "particles") in an ordered array. The particles are, in some embodiments, elements which will create a detectable signal in the presence of an analyte. The particles may produce optical (e.g., absorbance or reflectance) or fluorescence/phosphorescent signals upon exposure to an analyte. Examples of particles include, but are not limited to

functionalized polymeric beads, agarous beads, dextrose beads, polyacrylamide beads, control pore glass beads, metal oxides particles (e.g., silicon dioxide (SiO_2) or aluminum oxides (Al_2O_3)), polymer thin films, metal quantum particles (e.g., silver, gold, platinum, etc.), and semiconductor quantum particles (e.g., Si, Ge, GaAs, etc.). A detector (e.g., a charge-coupled device "CCD") in one embodiment is positioned below the sensor array to allow for the data acquisition. In another embodiment, the detector may be positioned above the sensor array to allow for data acquisition from reflectance of the light off of the particles.

Light originating from the light source may pass through the sensor array and out through the bottom side of the sensor array. Light modulated by the particles may pass through the sensor array and onto the proximally spaced detector. Evaluation of the optical changes may be completed by visual inspection or by use of a CCD detector by itself or in combination with an optical microscope. A microprocessor may be coupled to the CCD detector or the microscope. A fluid delivery system may be coupled to the supporting member of the sensor array. The fluid delivery system, in some embodiments, is configured to introduce samples into and out of the sensor array.

In an embodiment, the sensor array system includes an array of particles. The particles may include a receptor molecule coupled to a polymeric bead. The receptors, in some embodiments, are chosen for interacting with analytes. This interaction may take the form of a binding/association of the receptors with the analytes. The supporting member may be made of any material capable of supporting the particles, while allowing the passage of the appropriate wavelengths of light. The supporting member may include a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity.

In an embodiment, the optical detector may be integrated within the bottom of the supporting member, rather than using a separate detecting device. The optical detectors may be coupled to a microprocessor to allow evaluation of fluids without the use of separate detecting components. Additionally, a fluid delivery system may also be incorporated into the supporting member. Integration of detectors and a fluid delivery system into the supporting member may allow the formation of a compact and portable analyte sensing system.

A high sensitivity CCD array may be used to measure changes in optical characteristics which occur upon binding of the biological/chemical agents. The CCD arrays may be interfaced with filters, light sources, fluid delivery and micromachined particle receptacles, so as to create a functional sensor array. Data acquisition and handling may be performed with existing CCD technology. CCD detectors may be configured to measure white light, ultraviolet light or fluorescence. Other detectors such as photomultiplier tubes, charge induction devices, photo diodes, photodiode arrays, and microchannel plates may also be used.

A particle, in some embodiments, possess both the ability to bind the analyte of interest and to create a modulated signal. The particle may include receptor molecules which posses the ability to bind the analyte of interest and to create a modulated signal. Alternatively, the particle may include receptor molecules and indicators. The receptor molecule may posses the ability to bind to an analyte o f interest. Upon binding the analyte of interest, the receptor molecule may cause the indicator molecule to produce the modulated signal. The receptor molecules may be naturally occurring or synthetic receptors formed by rational design or combinatorial methods. Some examples of natural receptors include, but are not limited to, DNA, RNA, proteins, enzymes, oligopeptides, antigens, and antibodies. Either natural or synthetic receptors may be chosen for their ability to bind to the analyte molecules in a specific manner.

In one embodiment, a naturally occurring or synthetic receptor is bound to a polymeric bead in order to create the particle. The particle, in some embodiments, is capable of both binding the analyte(s) of interest and creating a detectable signal. In some embodiments, the particle will create an optical signal when bound to an analyte of interest.

A variety of natural and synthetic receptors may be used. The synthetic receptors may come from a variety of classes including, but not limited to, polynucleotides (e.g., aptamers), peptides (e.g., enzymes and antibodies), synthetic receptors, polymeric unnatural biopolymers (e.g., polythioureas, polyguanidiniums), and imprinted polymers. Polynucleotides are relatively small fragments of DNA which may be derived by sequentially building the DNA sequence.

Peptides include natural peptides such as antibodies or enzymes or may be synthesized from amino acids. Unnatural biopolymers are chemical structure which are based on natural biopolymers, but which are built from unnatural linking units. For example, polythioureas and polyguanidiniums have a structure similar to peptides, but may be synthesized from diamines (i.e., compounds which include at least two amine functional groups) rather than amino acids. Synthetic receptors are designed organic or inorganic structures capable of binding various analytes.

In an embodiment, a large number of chemical/biological agents of interest to the military and civilian communities may be sensed readily by the described array sensors. Bacteria may also be detected using a similar system. To detect, sense, and identify intact bacteria, the cell surface of one bacteria may be differentiated from other bacteria, or genomic material may be detected using oligonucleic receptors. One method of accomplishing this differentiation is to target cell surface oligosaccharides (i.e., sugar residues). The use of synthetic receptors which are specific for oligosaccharides may be used to determine the presence of specific bacteria by analyzing for cell surface oligosaccharides.

In one embodiment, a receptor may be coupled to a polymeric resin. The receptor may undergo a chemical reaction in the presence of an analyte such that a signal is produced. Indicators may be coupled to the receptor or the polymeric bead. The chemical reaction of the analyte with the receptor may cause a change in the local microenvironment of the indicator to alter the spectroscopic properties of the indicator. This signal may be produced using a variety of signalling protocols. Such protocols may include absorbance, fluorescence resonance energy transfer, and/or fluorescence quenching. Receptor-analyte combination may include, but are not limited to, peptides-proteases, polynucleotides-nucleases, and oligosaccharides- oligosaccharide cleaving agents.

In one embodiment, a receptor and an indicator may be coupled to a polymeric resin. The receptor may undergo a conformational change in the presence of an analyte such that a change in the local microenvironment of the indicator occurs. This change may alter the spectroscopic

properties of the indicator. The interaction of the receptor with the indicator may be produce a variety of different signals depending on the signalling protocol used. Such protocols may include absorbance, fluorescence resonance energy transfer, and/or fluorescence quenching.

In an embodiment, the sensor array system includes an array of particles. The particles may include a receptor molecule coupled to a polymeric bead. The receptors, in some embodiments, are chosen for interacting with analytes. This interaction may take the form of a binding/association of the receptors with the analytes. The supporting member may be made of any material capable of supporting the particles, while allowing the passage of the appropriate wavelengths of light. The supporting member may include a plurality of cavities. The cavities may be formed such that at least one particle is substantially contained within the cavity. A vacuum may be coupled to the cavities. The vacuum may be applied to the entire sensor array. Alternatively, a vacuum apparatus may be coupled to the cavities to provide a vacuum to the cavities. A vacuum apparatus is any device capable of creating a pressure differential to cause fluid movement. The vacuum apparatus may apply a pulling force to any fluids within the cavity. The vacuum apparatus may pull the fluid through the cavity. Examples of vacuum apparatuss include pre-sealed vacuum chamber, vacuum pumps, vacuum lines, or aspirator-type pumps.

Further details regarding these systems can be found in the following U.S. patent applications, all of which are incorporated herein by reference: U.S. Patent Application Serial No. 09/287,248 entitled "Fluid Based Analysis of Multiple Analytes by a Sensor Array"; U.S. Patent Application Serial No. 09/354,882 entitled "Sensor Arrays for the Measurement and Identification of Multiple Analytes in Solutions"; U.S. Patent Application Serial No. 09/616,355 entitled "Detection System Based on an Analyte Reactive Particle"; U.S. Patent Application Serial No. 09/616,482 entitled "General Signaling Protocols for Chemical Receptors in Immobilized Matrices"; U.S. Patent Application Serial No. 09/616,731 entitled "Method and Apparatus for the Delivery of Samples to a Chemical Sensor Array"; U.S. Patent Application Serial No. 09/775,342 entitled "Magnetic-Based Placement and Retention of Sensor Elements in a Sensor Array"; U.S. Patent Application Serial No. 09/775,340 entitled "Method and System for Collecting and Transmitting Chemical Information"; U.S. Patent Application Serial No.

09/775,344 entitled "System and Method for the Analysis of Bodily Fluids"; U.S. Patent Application Serial No. 09/775,353 entitled "Method of Preparing a Sensor Array"; U.S. Patent Application Serial No. 09/775,048 entitled "System for Transferring Fluid Samples Through A Sensor Array" (Published as U.S. Publication No.: 2002-0045272-A1); U.S. Patent Application Serial No. 09/775,343 entitled "Portable Sensor Array System"; and U.S. Patent Application Serial No. 10/072,800 entitled "Method and Apparatus for the Confinement of Materials in a Micromachined Chemical Sensor Array".

Method of Testing for Microbes Using A Membrane System

Shown in FIG. 1 is a device for measuring optical characteristics of solid analytes. Analyte detection device 2200 uses a porous membrane to capture solid analytes. Device 2200 includes a porous membrane 2210 coupled to a membrane support 2220. Membrane 2210 and membrane support 2220 may be held in a body 2230 between a top insert 2240 and bottom insert 2242 to form device 2200. Top insert 2240 may include window 2244 which allows viewing of an upper surface of membrane 2210 while the membrane is disposed in body 2230. Inserts 2240, 2242 and membrane support 2220 may be retained by a cap 2254 (depicted in cross section in FIG. 1). Cap 2254 may have external threads that engage complementary internal threads on body 2230. A spacer 2256 may be disposed between cap 2254 and top insert 2240.

Device 2200 also includes a fluid inlet 2260 and a fluid outlet 2270. Fluids for analysis may be introduced into device 2200 via fluid inlet 2260. Fluids exit fluid inlet 2260 and enter a first cavity 2262, which is defined by membrane support 2220, membrane 2210 and top insert 2240. The fluids may pass through membrane 2210 and into a second cavity 2272, which is defined by membrane support 2220, membrane 2210 and bottom insert 2242. Fluids may then exit the device via fluid outlet 2270.

Membrane 2210 is selected from a material capable of filtering the analytes of interest from a fluid stream. For examples, if microbes represent the analyte of interest, the membrane should be capable of removing microbes from a fluid stream. A suitable membrane may include a plurality of pores that have a size significantly less than the size of the analyte of interest. For

airborne toxic microbes (e.g., anthrax), the filter may be configured to capture microbes that have a diameter of greater than about 1 μm. It is believed that microbes that have a diameter of less than about 1 μm are very difficult to generate in large quantities, and if the organisms are viable, environmental stresses tend to interfere with the action of the microbes due to the high surface area/mass ratio. Membranes may be formed from a variety of materials known in the art. In one embodiment, membrane 2210 may be a track-etched Nuclepore™ polycarbonate membrane. A Nuclepore membrane is available from Whatman plc. Membrane 2210 may be about 5-10 microns in thickness. Membrane 2210 includes a plurality of pores. Pores may range from about 0.2 μm in diameter up to about 12 μm in diameter to capture potentially dangerous microbes.

In some embodiments, a detector may be used to analyze the analytes that are collected by the membrane device 2200. As depicted in FIG. 2, a detector may be placed over a portion of device 2200 such that an image of the membrane may be captured by the detector. For example, detector may be placed such that images 2210 of the membrane may be taken through window 2244. Detector 2280 may be used to acquire an image of the particulate matter captured on membrane 2210. Image acquisition may include generating a "digital map" of the image. In an embodiment, detector 2280 may include a high sensitivity CCD array. The CCD arrays may be interfaced with filters, light sources, fluid delivery, so as to create a functional sensor array. Data acquisition and handling may be performed with existing CCD technology. In some embodiments, the light is broken down into three color components, red, green and blue. Evaluation of the optical changes may be completed by visual inspection (e.g., with a microscope) or by use of a microprocessor 2290 coupled to the detector. For fluorescence measurements, a filter may be placed between detector 2280 and membrane 2210 to remove the excitation wavelength.

In an alternate embodiment, a membrane based analyte detection device may include additional outlets and control valves to offer additional fluid control paths in the device. uses a porous membrane to capture solid analytes, as depicted in FIG. 3. Device 2205 includes a porous membrane 2210 coupled to a membrane support 2220. Membrane 2210 and membrane support

2220 may be held in a body 2230 between a top insert 2240 and bottom insert 2242 to form device 2205 as disclosed previously. Device 2205 also includes a fluid inlet 2260 and a first fluid outlet 2270 and a second fluid outlet 2274. Device 2205 also includes inlet valve 2290, first outlet valve 2292 and second outlet valve 2294. Inlet valve 2290 is configured to control the flow of a fluid stream through fluid inlet 2260. First outlet valve 2292 is configured to control the flow of a fluid stream through first fluid outlet 2270. Second outlet valve 2294 is configured to control the flow of a fluid stream through second fluid outlet 2274.

Device 2205 may be operated in two different modes based on which valves are opened. A configuration of device 2205 in a "flow through" mode is depicted in FIG. 3. In this mode, fluid is passed through the membrane to allow capture of analytes or the addition of development agents. Fluids for analysis may be introduced into device 2205 via fluid inlet 2260. Fluid inlet valve 2290 is placed in an "open" position to allow the flow of fluid through fluid inlet 2260. Fluids exit fluid inlet 2260 and enter a first cavity 2262, which is defined by membrane support 2220, membrane 2210 and top insert 2240. During a "flow through" operation, second outlet valve 2294 is placed in a "closed" position to inhibit the flow of fluid through second fluid outlet 2274. The fluids may, therefore, be forced to pass through membrane 2210 and into a second cavity 2272, which is defined by membrane support 2220, membrane 2210 and bottom insert 2242. Fluids may then exit the device via first fluid outlet 2270. First outlet valve 2292 is placed in an "open" position to allow the flow of fluid through first fluid outlet 2270.

Device 2205 may also be operated in a "lateral membrane wash" mode, as depicted in FIG. 4. In this mode, the membrane is cleared by the passage of a fluid across the collection surface of the membrane. This allows the membrane to be reused for subsequent testing. Fluids for waching the membrane may be introduced into device 2205 via fluid inlet 2260. Fluid inlet valve 2290 is placed in an "open" position to allow the flow of fluid through fluid inlet 2260. Fluids exit fluid inlet 2260 and enter a first cavity 2262, which is defined by membrane support 2220, membrane 2210 and top insert 2240. During a "lateral membrane wash" operation, first outlet valve 2292 is placed in a "closed" position to inhibit the flow of fluid through first fluid outlet 2270. The closure of first outlet valve inhibits the flow of fluid through membrane 2210.

The fluids may, therefore, be forced to exit the device through second fluid outlet 2274. Second outlet valve 2294 is placed in an "open" position to allow the flow of fluid through second fluid outlet 2274. Since fluid is inhibited from flowing through the membrane, any analytes and other particles collected by the membrane may be "washed" from the membrane to allow further use.

In an alternate embodiment, a device 2205 (or device 2200) may be subjected to a "back flush" treatment. For either device, the fluid outlet 2270 is used to introduce a fluid into the device while fluid inlet 2260 is used to allow the fluid to exit the device. This "reverse" flow of fluid through the cell allows the membrane to be cleared. For device 2205, the valves will be configured as in FIG. 3, with the washing fluid being introduced through first fluid outlet 2270. Either a lateral membrane wash or a back flush treatment may be used to clear analytes and other particles from a membrane.

A schematic diagram of an analyte detection system 2300 that includes a membrane based analyte detection device 2205 is depicted in FIG. 5. While the following description is directed to the use of device 2205, it should be understood that detection device 2200 may also be used in such a system. Analyte detection device 2205 is coupled to the other analyte detection system components via fluid inlet 2260, first fluid outlet 2270 and second fluid outlet 2274. Fluid inlet 2260 is coupled to first pump 2360. First pump 2360 is configured to deliver fluids to the analyte detection device 2205 through fluid inlet 2260. First pump 2360 may be a peristaltic pump. First pump may also be configured to pressurize the fluid stream being sent to device 2205. This may help to increase the flow rate of fluid through device 2205. First pump 2360 may also be coupled to a manifold 2320. Manifold 2320 is configured to couple first pump 2360 to a variety of fluid containers 2310. Fluid containers 2310 may include samples to be tested, buffer fluids, and visualization fluids. Some fluid containers may also be used for waste collection during membrane washing steps.

System 2205 may also include a second pump 2365. Second pump may be coupled to a multi-valve switch 2367. Multi-valve switch may allow fluids to be drawn into the system for pumping by first pump 2360. If a fluid container is not in use, air may collect in the fluid

container and the tubing connecting the fluid container to the manifold. Prior to use, this air may be removed from the system by coupling second pump 2365 to manifold 2330 and operating the second pump to "prime" the system for use. After any air is removed from the tubing, the multi-valve switch may be switch to disconnect second pump 2365 from the system (as depicted in FIG. 5) and reconnect first pump 2360 to the manifold.

Additional fluid containers may be coupled to the outlets of analyte detection device 2205. In one embodiment, a fluid container 2370 may be used to collect effluent waste from the detection device. Additionally, a fluid container 2372 may include a washing fluid (e.g., a buffer solution) to use for during back flush operations. Analyte detection system 2300 also includes valves 2280, 2282, and 2284 to control flow of liquids through the system.

FIG. 5 depicts a configuration of system 2300 that may be used for the collection of samples. Manifold 2320 may be operated to select the appropriate fluid container 2310 for analysis. Second pump 2365 may be used to draw the fluid from a fluid container 2310 into the manifold and to draw out any air that is present in the connecting tubing. After the system is primed, first pump 2360 is used to convey fluid from the selected fluid container to the analyte detection device. Arrows in FIG. 5 depict the flow of fluid from manifold 2320 to pump 2360. Pump 2360 sends the fluid from manifold 2320 to analyte detection device 2205, where a sample is collected. Fluid exits analyte detection device 2205 through fluid outlet 2270. Fluid outlet 2270 is coupled to a waste receptacle 2370 where the waste fluid is collected. Valve 2280 is placed in an open position, while valves 2282 and 2284 are kept in a closed position to allow the flow of the effluent to fluid container 2370. It should also be understood that the configuration depicted in FIG. 5 may also be used to add visualization agents to the collected sample and for lateral flow through, where the manifold is adjusted to a fluid container that includes a visualization agent or a washing fluid as appropriate.

FIG. 6 depicts a configuration of system 2300 that may be used for a back flush operation of a membrane. In the configuration depicted in FIG. 6, the flow of fluid is reversed to flush the membrane. To switch directions, pump 2360 is operated in the reverse direction then depicted in

FIG. 5. Pump 2360 draws fluid from the analyte detection device toward manifold 2320. Manifold 2320 directs the fluid to an appropriate container for collecting the effluent from the detection device. A washing fluid (e.g., a buffer solution) is sent through detection device 2205 from fluid container 2372. Valve 2282 is kept in an open position to allow fluid to flow from container 2372 to detection device 2205. Valves 2280 and 2284 are placed in a closed position. The fluid being pulled through analyte detection device will pass through the membrane and remove particles that have been trapped by the membrane to allow further testing.

Analyte detection system may be used to determine the presence of analytes in a fluid system. One embodiment of a process for determining analytes in a fluid sample is depicted in the flow chart of FIG. 7. Prior to the analysis of any samples, a background sample may be collected and analyzed. Solid analytes are typically collected and stored in a liquid fluid. The liquid fluid that is used to prepare the samples, may be analyzed to determine if any analytes are present in the fluid. In one embodiment, a sample of the liquid fluid used to collect the solid analytes is introduced into an analyte detection device 2205 to determine the background "noise" contributed by the fluid. Any particles collected by the membrane during the background collection are viewed to determine the level of particulate matter in the liquid fluid. In some embodiments, particles collected by the membrane during the collection stage may be treated with a visualization agent to determine if any analytes are present in the liquid fluid. The information collected from the background check may be used during the analysis of collected samples to reduce false positive indications.

After collection of the background sample, the membrane may be cleared using either a back flush wash or a lateral wash, as described herein. After clearing the membrane, the system may be used to analyze samples for solid analytes (e.g., microbes). As used herein the term "microbes" refers to a variety of living organisms including bacteria, spores, viruses, and protozoa. As the collected sample is passed through the porous membrane, the porous membrane traps any particles that have a size that is greater than the size of the pores in the porous membrane. Collection of particles may be continued for a predetermined time, or until all of the collected sample has been passed through the membrane.

After collection, the particles collected by the membrane may be analyzed using a detector. In some embodiments, the detector may be a camera that will capture an image of the membrane. For example, a detector may be a CCD camera. Analysis of the particles captured by the membrane may be performed by analyzing the size and/or shape of the particles. By comparing the size and/or shape of the particles captured by the membrane to the size and shape of known particles the presence of a predetermined analyte may be indicated. Alternatively, microbe analytes will react to a variety of visualization agents (e.g., colored and fluorescent dyes). In one embodiment, the detection of microbe analytes may be aided by the staining of the microbe with a visualization agent. The visualization agent will induce a known color change or impart fluorescence to a microbe. In an embodiment, particles captured by the membrane are stained and the particles analyzed using an appropriate detector. The presence of particles that have the appropriate color and/or fluorescence may indicate the presence of the analyte being tested for. Typically non-microbe particles (e.g., dust) will not undergo the same color and/or fluorescent changes that microbes will when treated with the visualization agent. The visualization agent may include a "cocktail" mixture of semi-specific dyes, which may be designed to mark microbes of interest. Selection of the mixture may be based on the capacity of the dye chromophore to create an optical fingerprint that can be recognized by a detector and associated imaging software as being associated with specific pathogenic bacteria or spores, while at the same time distinguishing from the signal exhibited by dust and other background particulate matter.

The analysis of the particles may indicate that an analyte of interest is present in the sample. In this case, the particles may be flushed from the membrane and sent out of the system for further testing. Further testing may include techniques such as cultures or ELISA techniques that may allow more accurate determination of the specific analytes present. Alternatively, the particles may be sent to a sensor array, as described herein, for further testing. If no significant amounts of analytes are found on the membrane, the membrane may be washed and other samples analyzed.

In an embodiment, user-defined threshold criteria may be established to indicate a probability that one or more specific microbes are present on the membrane. The criteria may be based on one or more of a variety of characteristics of the image. In some embodiments, the criteria may be based on pixel or color fingerprints established in advance for specific microbes. The characteristics that may be used include, but are not limited to, the size, shape, or color of portions of matter on the image, the aggregate area represented by the matter, or the total fluorescent intensity of the matter. In an embodiment, the system may implement an automated counting procedure developed for one or more pathogenic and non-pathogenic bacteria.

In an embodiment, the membrane system may include a computer system (not shown). Computer system may include one or more software applications executable to process a digital map of the image generated using detector. For example, a software application available on the computer system may be used to compare the test image to a pre-defined optical fingerprint. Alternatively, a software application available on computer system may be used to determine if a count exceeds a pre-defined threshold limit.

A detector may be used to acquire an image of the analytes and other particulate matter captured on a membrane. Microbes may collect on a membrane along with dust and other particulate matter and be captured in an image produced from a detector. The image acquired by the detector may be analyzed based on a pre-established criteria. A positive result may indicate the presence of a microbe. The test criteria may be based on a variety of characteristics of the image, including, but not limited to, the size, shape, aspect ratio, or color of a portion or portions of the image. Applying test criteria may allow microbes to be distinguished from dust and other particulate matter. During analysis, the flow of sample through from a fluid delivery system may be continued.

In some embodiments, a positive result may create a presumption that the fluid contains a particular analyte. If the image yields a positive result with respect to the test criteria, a sample of the fluid may be subjected to a confirmatory or specific testing. On the other hand, if the

image yields a negative result with respect to the test criteria, membrane may be rinsed and the preceding method may be carried out for fluid from another sample.

During analyte testing a sample may be introduced into the analyte detection device. A trigger parameter may be measured to determine when to introduce the visualization agent into the analyte detection device. Measurement of the trigger parameter may be continuous or may be initiated by a user. Alternatively, the stain may be introduced into the analyte detection device immediately after the sample is introduced.

In one embodiment, the trigger parameter may be the time elapsed since initiation of introducing the fluid into an analyte detection device at a controlled flow rate. For example, the stain may be introduced 20 seconds after initiation of introducing the fluid sample into an analyte detection device at a flow rate of 1 milliliter per minute. In another embodiment, the trigger parameter may be the pressure drop across the membrane. The pressure drop across the membrane may be determined using a pressure transducer located on either side of the membrane.

In another embodiment, the trigger parameter may be the autofluorescence of analytes captured by the membrane. A detector may be switched on until a pre-defined level of signal from the autofluorescence of the analytes has been reached. In still another embodiment, filtering software may be used to create a data map of the autofluorescence of the matter on the membrane that excludes any pixels that contain color in a blue or red spectral range. The data map may be used to compute a value for particles that are autofluorescent only in the "pure green" portion of the visible spectrum.

In some embodiments, a presumptive positive result may be inferred if the trigger parameter exceeds a certain value without applying a stain. For example, a presumptive positive result may be inferred where the autofluorescence value is more than twice the value that would

indicate application of a stain. In such a case, the application of a stain may be dispensed with and a confirmatory test may be conducted for the sample.

If the value of the trigger parameter is less than would indicate proceeding directly to the confirmatory test, but exceeds the value established to trigger the application of a stain, then a stain may be introduced into an analyte detection device.

Collecting a sample of a fluid may include gathering a sample from a solid, liquid, or gas. In some embodiments, the sample may be derived from collecting air from a target environment in an aerosol form, then converting aerosol into a hydrosol. For example, particles from 500 liters of an air sample may be collected deposited into about 0.5 milliliters of liquid. U.S. Patent No. 6,217,636 to McFarland, entitled "TRANSPIRATED WALL AEROSOL COLLECTION SYSTEM AND METHOD," which is incorporated herein by reference as if fully set forth herein, describes a system for collecting particulate matter from a gas flow into a liquid using a porous wall.

In one embodiment, a system as described above, may be used to determine the presence of anthrax spores or bacteria. Collection of air samples in a potentially contaminated area may be concentrated in a fluid sample using an airsol collector. The fluid sample may be passed through a membrane based detector system as described herein. The membrane based detection system may collect any particle collected by the airsol collector. The particles collected may be treated with a visualization agent that includes stains that are specific for anthrax bacteria. Such visualization agents are know to one of ordinary skill in the art. The presence of particles that exhibit the appropriate color/fluorescence may indicate that anthrax is presence. The indication of anthrax may be further determined by additional confirmation testing.

Pixel Analysis Methods for Detection of Microbes

In some embodiments, pixel analysis methods may be used in the analysis of an image of a fluid or captured matter. For example, pixel analysis may be used to discriminate microbes from

dust and other particulate matter captured on a membrane. Pixel analysis may include analyzing characteristics of an image to determine whether a microbe is present in the imaged fluid.

Pixel analysis may be based on characteristics including, but not limited to, the size, shape, color, and intensity ratios of an image or portions of an image. As an example, the total area that emits light in an image may be used to conduct analysis. As another example, the green fluorescent intensity of an image may be used to conduct analysis. In an embodiment, an "optical fingerprint" for a specific microbe or set of microbes may be established for use in pixel analysis. In some embodiments, pixel analysis may be based on ratios between values, such as an aspect ratio of an element of matter captured on an image. In other embodiments, pixel analysis may be based on threshold values.

During use, a visualization agent may cause different particles to emit different wavelengths of light depending on the nature of the particle. When the particles are analyzed with a camera, a user may be able to determine if a particular analyte is present based on the color of the particle. For example, a green particle may indicate the presence of an analyte of interest. Any other colored particles may not be of interest to a user. While a person may be able to discern between colors, it is desirable for a computer system to also be able to discern different colors from a membrane sample. Many detectors can only discern specific colors when analyzing an image. For example, many CCD detectors can only discern red, blue and green colors. Thus, a CCD detector may not be able to discern the difference between a particle that emits both blue and green light, although the color difference may be apparent to a person using the system. To overcome this problem a method of subtracting out particles having the "wrong" color may be used.

In some embodiments, pixels of an image that do not fall within a color range specified by a user may be discarded from the image. In one embodiment, a fluid may be stained to cause a microbe of interest to emit light in only the green portion of the visible spectrum. By contrast, dust and other particles contained in the fluid may emit light in combinations of green, blue, and red portions of the visible spectrum in the presence of the stain. To isolate the portion of the

image that represents only the microbe of interest, binary masks may be created to eliminate light emissions caused by non-microbial matter from the image.

Such a method is depicted in FIGS 8A-E. FIG. 8A shows an image of all particles captured by a membrane. In an embodiment, an image is then captured of only the particles that exhibit color in the red portion of the spectrum (See FIG. 8B). The image of "red" particles is used to create a mask that may be compared to the full spectrum view of the particles. Since the analytes of interest only exhibit color in the green portion of the spectrum, any particle with color in the red portion of the spectrum may be removed from the original image. FIG. 8C shows the original image but with the particles that appear in the red portion of the spectrum subtracted from the image. The remaining particles are potential particles that may be the analyte of interest.

In a second iteration FIG. 8D shows a binary mask that may be used to mask any pixels that include a blue component. An image is captured of only the particles that exhibit color in the blue portion of the spectrum (See FIG. 8D). The image of "blue" particles is used to create a mask that may be compared to the full spectrum view of the particles. Since the analytes of interest only exhibit color in the green portion of the spectrum, any particle with color in the blue portion of the spectrum may be removed from the original image. FIG. 8E shows the original image but with the red binary mask and blue binary mask applied so that pixels including a red or blue component are excluded. The particles that remain in the image are thus particles that only exhibit a green color. Thus, the method may be used to produce an image that includes only "pure green" pixels. Such an image may be analyzed to detect the presence of a microbe by eliminating particles that are not relevant. It should be understood that while the above recited example is directed to determining the presence of green particles it should be understood that the process can be modified to determine blue particles only, red particles only, or particles that exhibit combinations of colors (e.g., red and blue, red and green, blue and green, or red, blue and green).

In some embodiments, pixel analysis may be used in combination with the membrane method for detecting a microbe described herein. Pixel analysis may be conducted either before

or after the application of a stain. In an embodiment, pixel analysis may be used to determine when to apply a stain.

After an analyte of interest is detected using a membrane based detection device further testing may be performed to identify the analyte. In one example, the particles captured by the membrane may be transferred to a sensor array as described in any of the following U.S. Patent Applications: U.S. Patent Application Serial No. 09/287,248 entitled "Fluid Based Analysis of Multiple Analytes by a Sensor Array"; U.S. Patent Application Serial No. 09/354,882 entitled "Sensor Arrays for the Measurement and Identification of Multiple Analytes in Solutions"; U.S. Patent Application Serial No. 09/616,355 entitled "Detection System Based on an Analyte Reactive Particle"; U.S. Patent Application Serial No. 09/616,482 entitled "General Signaling Protocols for Chemical Receptors in Immobilized Matrices"; U.S. Patent Application Serial No. 09/616,731 entitled "Method and Apparatus for the Delivery of Samples to a Chemical Sensor Array"; U.S. Patent Application Serial No. 09/775,342 entitled "Magnetic-Based Placement and Retention of Sensor Elements in a Sensor Array"; U.S. Patent Application Serial No. 09/775,340 entitled "Method and System for Collecting and Transmitting Chemical Information"; U.S. Patent Application Serial No. 09/775,344 entitled "System and Method for the Analysis of Bodily Fluids"; U.S. Patent Application Serial No. 09/775,353 entitled "Method of Preparing a Sensor Array"; U.S. Patent Application Serial No. 09/775,048 entitled "System for Transferring Fluid Samples Through A Sensor Array" (Published as U.S. Publication No.: 2002-0045272-A1); U.S. Patent Application Serial No. 09/775,343 entitled "Portable Sensor Array System"; and U.S. Patent Application Serial No. 10/072,800 entitled "Method and Apparatus for the Confinement of Materials in a Micromachined Chemical Sensor Array".

FIG. 9 depicts a system in which a particle sensor array detector 2380 is coupled to a membrane analyte detection device 2205. Membrane based analyte detection device may be part of an analyte detection system as previously described. After a sample is passed through a membrane, the particles collected by the membrane may be subjected to an additional test to further identify the analytes. In one embodiment, the analytes may be washed from the surface o the membrane and transferred to a sensor based analyte detection system, as described in any of

the previously referenced patent applications. The analytes extracted from the sample may react with beads that are placed in a sensor array. The reaction of the analytes with the sensor array beads may allow confirmation (or further identification) of the analytes. Methods of detecting microbes using a sensor array system are described in further detail in the above-referenced patent applications.

Many microbes may not react with a bead of a sensor array. Large microbes may be unable to make proper contact with the bead and therefore are not detected by the bead. In one embodiment, the microbes are subjected to a treatment that allows better detection by a bead based detection system. In one embodiment, the particles may be subjected to lysis conditions. Lysis of microbes will cause the disintegration or dissolution of the microbe. For bacteria, lysis may be induced by treatment with an alkali base or by use of enzymes. Lysis of the bacteria allows portions of the material contained by the bacteria to be released and analyzed. Typically, either proteins or nucleic acids released from the bacteria may be analyzed.

Microbes such as bacteria, spores, and protozoa in a fluid may be captured in the macropores of the beads. In some embodiments, receptors, including, but not limited to, selective antibodies or semi-selective ligands such as lectins, may be coupled to a particle in an internal pore region of the particle to create a selective bead. Suitable receptors may be selected using the methods described herein. In some embodiments, a visualization antibody may be introduced that may couple with the captured analyte. The visual antibody may yield a colorimetric or fluorescence signature that can be recorded by the CCD detector. In some embodiments, a series of selective and semi-selective beads may be used in conjunction with the sensor array system described herein.

In an embodiment, an agent that is known to bind or interact with a microbe may be introduced into a fluid prior to the time that the microbes are placed in proximity with a sensor array. Such agents may have characteristics that facilitate capture of a microbe by a particle in the sensor array.

In an embodiment, a particle having macropores may be formed of agarose. A depiction of such a particle is shown in FIG. 10. A particle may be in the form of a spherical bead. The particle may include a plurality of macropores on its surface and interior.

In an embodiment, agarose may be used as a starting material for a macroporous particle because it is biocompatible and may be capable of interacting with biomolecules and living organisms. Activated agarose may demonstrate an affinity interaction with bacteria and microorganisms. To facilitate this interaction, specific properties on particles may be used to target specific microorganisms or cells. Processed agarose, in which sulfate groups have been eliminated from the agarose chain, may consist of an uncharged hydrophilic matrix with primary and secondary alcohols that can be used for activation and attachment. For example, the chemical surface of particles may be modified by oxidizing adjacent diols into aldehyde groups. Using sodium meta-periodate ($NaIO_4$) aliphatic aldehydes may be obtained that can be used in reductive amination coupling procedures.

In an embodiment, macroporous particles may be formed by suspension polymerization using a gel. Size, shape, and uniformity of the particle may depend on the hydrophilic/hydrophobic additives used to stabilize the emulsion. Pore size may be determined by agarose concentration of the gel. Mechanical properties, such as gel strength, may be affected by the molecular weight of the agarose. In one embodiment, suspension polymerization may be accomplished using a biphasic system containing the agarose monomer and emulsion stabilizers. A dispersion of a hydrophilic emulsifier (such as TWEEN 85) in cyclohexane may be added to a stirring aqueous solution of agarose at 60°C for 5 min to produce an oil-in-water emulsion. Fine particles of agarose stabilized by the emulsifier may be formed in this step. Next, a solution of a hydrophobic emulsifier (such as SPAN 85) may be added to the first emulsion forming a water-in-oil emulsion. Afterwards, the water-in-emulsion may be cooled to room temperature. Polymeric particles may appear at about 40°C. The aggregation of droplets, which may be referred to as flocculation, may form a matrix with oil droplets that will form pores or conduits in the beads. The particles may be washed with distilled water and alcohol, sized with industrial sieves, and preserved in water.

Emulsifiers added to the hydrophilic and/or hydrophilic phases and the concentration of the agarose solution may influence the quality of the beads. Additionally, mixing speed, nature of the agitation, and temperature during the preparation process may be important. The stability of the solutions may depend on the selected emulsifiers and the solvents used.

A particle may be of a substantially spherical shape. Particles with spherical geometry may enhance the available area for surface interaction with the analytes. Creating pores within the particles may also increase surface area. Particles may have large connecting flow pores in addition to normal diffusion pores. A macroporous particle may have improved mass transfer properties compared to a non-macroporous particle.

A particle may have a diameter of between about 250-300 microns. Macropores in a particle may be less than about 1 micron. Different pore sizes and shapes may allow for the entrapment and detection of a variety of analytes, including, but not limited to, cells, bacteria, DNA oligomers, proteins/antibodies, and small molecules.

An alternative process to suspension polymerization may be the use of a foaming agent to vary the porosity of the particles. For example, the decomposition of azides or carbonates during polymerization may allow incorporation of nitrogen or carbon dioxide "bubbles" into the particles. Because the gelling point for agarose is 40°C, the decomposition reaction should be performed at low temperatures.

Another alternative to suspension polymerization may be the use of molecular imprinting. The imprinting of particles may occur by non-covalent and covalent methods. Non-covalent imprinting may be based on non-covalent interactions such hydrogen bonds, ionic bonds, and Van der Waals forces between functional monomer and a template. The stability of the monomer-template complex prior to polymerization may depend on the affinity constants between the template and the functional monomers. In the covalent method, the bonds formed

between the functional monomer and the template may be cleaved once the polymerized matrix is obtained.

Within the covalent and non-covalent based approaches, there may be different methods for making molecular imprinted polymers. One approach may involve grinding the imprinted polymer to reduce their size to approximately 25 μm and expose the imprinted surfaces. Another technique, which may be referred to as 'surface template polymerization,' uses water and oil. In this technique, the water-soluble template may interact with the functional monomer at the water-oil interface. The complex monomer-template in the organic phase may be polymerized yielding a polymer-imprinted surface. This technique may allow the imprinting of water-soluble substances like zinc ions.

Other methodologies for imprinting polymers may be suitable. Molecular imprinting on microgel spheres may be a convenient procedure for imprinting agarose because the imprinted gel does not need to be reduced in size by grinding as in conventional molecular imprinting. Discrete imprinted microgels and imprinted microspheres may be synthesized by cross-linking polymerization of the monomer in the presence of the template, a process known as "precipitation polymerization."

Surface template polymerization and precipitation polymerization may be suitable alternative techniques to chemical surface modification of regular particles. Both techniques may be suitable for imprinting agarose with such templates as bacterial spores. A chromatography column mounted with imprinted beads may be a fast method for evaluating the efficacy of the imprinted beads. For example, bacteria may be re-bound, exposed to the fluorescent calcium-sensitive indicator known as calcein, and detected by fluorescence spectroscopy.

Molecular imprinting may allow the exploitation of organisms as reactors. The pores in the particle may facilitate feeding of entrapped microorganism reactants and cause them to produce a desired product. Molecular imprinting may be used for encapsulating bacteria such as

the Rhizobium organisms into agarose. These bacteria may convert nitrogen from the atmosphere into ammonia. By "feeding" these bacteria nitrogen, ammonia may be produced. The pores encapsulating the bacteria may retain an imprint of the organism for morphologic studies of the bacteria's surface.

High-performance liquid chromatography and fluorescent assays may be a valuable tool for studying the molecularly imprinted polymers. The fluorescent dye acridine orange may stain agarose beads so they may be morphologically analyzed with confocal scanning laser microscopy. Other morphological studies include atomic force microscopy, scanning electron microscopy, and microtome techniques. Characterization of the surface area of the beads, may be achieved by measuring the adsorption isotherm and using the Brunauer, Emmet, and Teller equation.

In some embodiments, the surface of a particle may be chemically modified. In other embodiments, chemical functionality, including, but not limited to non-specific (i.e., functional groups) and highly specific (i.e., bio-ligands such as antibodies) may be localized into the confines of the pore region. Chemical functionality may facilitate the entrapment of a variety of analytes.

In an embodiment, a particle may include a receptor that includes a particular metal. The metal may be capable of binding a material that is characteristic of a particular analyte. For example, a particle may be formed that includes terbium (III). Terbium (III) forms a luminescent complex with dipicolinic acid, a substance unique to spores.

Example:

Macroporous beads were prepared using the method for biphasic suspension polymerization method described herein. The beads so obtained were analyzed using light and fluorescence microscopy. The transparency of the agarose beads permitted the visualization of the fluorescent beads in different sections of the agarose beads. The presence of pores was

confirmed by adding 1 μm fluorescent beads. Using light and fluorescence microscopy, the presence of conduits could not be conclusively determined. The beads accumulated into voids present in the bead, probably the ends of conduits.

Experiments were initially performed using Merck's Omnipure agarose powder. Low yields of non-spherical particles ranging between 250 and 300 μm were obtained. Experiments performed with an exaggerated amount of the hydrophilic emulsifier, 3.5 mL span 85 resulted in beads without pores but with a rough surface. By reducing the amount of the hydrophobic emulsifier, massive gellation due to the poor stabilization of the agarose particles in the oil in water emulsion occurred.

Agarose aqueous solution concentration 4% (w/v),
o/w emulsion: 0.7mL tween 80/10 mL cyclohexane
w/o emulsion: 7 mL span 85/75 mL cyclohexane

Stirring speed with a magnetic stirrer	Fluorescence and light microscopy	Apparent porosity	Efficiency Size 250-300 μm
10	With oil inclusions, regular integrity	A few	Less than 10%
9	Medium integrity	None	About 10%
8	Better integrity	A few but more than stir at 10	About 10%

Table 1. Effect of the stirring speed on the fabrication of porous agarose beads

The effect of stirring speed has been briefly evaluated. With higher stirring speeds the integrity of the beads was poor. Smaller particles are expected to be the result of faster stirring speeds, but exposure to higher physical stress only results in the disintegration of the beads. Trials performed under the same conditions using Sigma agarose gave similar results to Merck

agarose, but with slightly higher yields around 20%. The integrity of the beads improved slightly suggesting better mechanical properties such as gel strength.

Experiments for producing homogeneous particles were performed using agarose obtained from Merck at a constant concentration of agarose solution and stirring. The results are shown in table 2.

Agarose aqueous solution concentration 4% (w/v),
o/w emulsion: 0.7mL tween 80/10 mL cyclohexane
w/o emulsion: 7 mL span 85/75 mL cyclohexane

Stirring speed with a magnetic stirrer	Fluorescence and light microscopy	Efficiency Size 250-300 μm
10	Opaque beads	**About 10%**
10	Regular integrity	**About 10%**
10	**Bad integrity**	**Less than 10%**

Table 2. Effect of the emulsifier on the fabrication of homogeneous agarose beads

Excessive stabilization of the water in oil emulsion causes reduced flocculation and increases the formation of fines resulting in a lower yield. Performing the same experiment with a fixed stirrer speed of 8 (Corning stirrer/hot plate, model # PC-420) slightly increased the yield. Magnetic stirring may not be appropriate for viscous solutions or the foam obtained during emulsification (creaming).

Bead Selection Techniques

Sensor arrays that use beads (either non-porous or porous) can be used to determine the presence of a variety of analytes. Typically, the beads include a receptor that binds to an analyte. The receptor may also bind to an indicator. The indicator typically produces a signal in the

presence of an analyte that is different from a signal produced in the absence of an analyte. The selection of beads for use with a particular analyte may be important to the success of the sensor array. In general, a bead should have a high affinity for the analyte and produce an easily detectable signal. A method is described herein which may be used to determine an optimal receptor for a given analyte and indicator.

One method used to determine the presence of an analyte is a displacement assay. In a displacement assay a bead that includes a receptor is preloaded with an indicator. The indicator interacts (e.g., is bound to) the receptor such that the bead appears to have a specific color or fluorescence due to the indicator. When a solution that includes an analyte is brought into contact with the bead, the analyte may displace the indicator from the receptor. This displacement may cause a loss of color or fluorescence of the bead since the indicator is no longer associated with the bead. By measuring the loss of color or fluorescence of the bead, the presence of an analyte may be determined. The success of such an assay for determining the presence of an analyte is dependent, in part, on the interaction of the receptor with the analyte and the indicator. Generally, the bead should show a maximum color and fluorescence when an indicator is bound to the receptor, however, the indicator should be easily displaced by the analyte.

In one embodiment, a plurality of beads having a variety of receptors may be produced. In one embodiment, the receptors may be formed from a variety of different receptor types. Alternatively, the beads may have similar receptors. For example, techniques are well known to create libraries of peptide, peptide mimics, or nucleotides upon polymeric beads. For peptide libraries up to 20^n different beads may be produced in a library, where n is the number of amino acids in the peptide chain. Nucleic acid libraries may have up to 4^n different beads where n is the number of nucleic acid bases. Because of the large number of different beads in these libraries, the testing of each individual bead is very difficult.

FIG. 11 depicts a schematic drawing of a method for optimizing a receptor on a bead. In FIG 11A, a bead is depicted that includes a receptor X. Receptor X is composed of 6 subparts

that extend from a base. The base is coupled to the bead. The bead is first contacted with an indicator, denoted as the stars in FIG. 11A. The indicator interacts with each of the beads in the library, binding to the receptors. FIG. 11B shows the indicator coupled to the receptor of the bead. As depicted in FIG. 11b, the color or fluorescence of the bead is altered due to the interaction of the indicator with the receptor. The change in color or fluorescence of the bead indicates that the bead is capable of interacting with the indicator.

When a plurality of beads is used, the indicator will bind to the beads at various strengths. The strength of binding is typically associated with the degree of color or fluorescence produced by the bead. A bead that exhibits a strong color or fluorescence in the presence of the indicator has a receptor that binds with the indicator. A bead that exhibits a weak or no color or fluorescence has a receptor that only weakly binds the indicator. Ideally, the beads which have the best binding with the indicator should be selected for use over beads that have weak or no binding with the indicator. FIG. 12 depicts a schematic of a flow cytometer which may be used to separate beads based on the intensity of color or fluorescence of the bead. Generally, a flow cytometer allows analysis of each individual bead. The beads may be passed through a flow cell that allows the intensity of color or fluorescence of the bead to be measured. Depending on the measured intensity, the bead may be collected or sent to a waste collection vessel, as indicated in FIG. 12. For the determination of an optimal bead for interaction with an indicator, the flow cytometer may be set up to accept only beads having an color or fluorescence above a certain threshold. Beads that do not meet the selected threshold, (i.e., beads that have weak or no binding with the indicator) are not collected and removed from the screening process. Flow cytometers are commercially available from a number of sources.

After the bead library has been optimized for the indicator, the beads that have been collected represent a reduced population of the originally produced beads. If the population of beads is too large, additional screening may be done by raising the intensity threshold. Now that the beads that exhibit optimal interaction with a receptor have been identified, the remaining beads are optimized for displacement of the indicator by the analyte of interest. Thus, the remaining beads are treated with a fluid that includes the analyte of interested, as depicted in

FIG. 11C. The analyte is represented by the circle. For some beads, the analyte will cause displacement of the indicator, causing the color or fluorescence of the bead to be reduced, as depicted in FIG. 11D. The intensity of the color or fluorescence of the bead after it interacts with an analyte will be based on how the competitive displacement of the indicator. A bead that exhibits weak or no color or fluorescence when treated with an analyte is the most desirable. Such beads show that the analyte is readily bound by the receptor and can readily displace the indicator from the receptor.

Once again a flow cytometer may be used to determine the optimal beads for use in an assay. A library of beads that have been optimized for interaction with an indicator are treated with a fluid that includes an analyte. The treated beads are passed through a flow cytometer and the beads are separated based on intensity of color or fluorescence. The beads that exhibit a color or fluorescence below a predetermined intensity are collected, while beads that show a color or fluorescence above the predetermined intensity are sent to a waste collection. The collected beads represent the optimal beads for use with the selected analyte and indicator. The identity of the receptor coupled to the bead may be determined using known techniques. After the receptor is identified, the bead may be reproduced and used for analysis of samples.

Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as the presently preferred embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements described herein without departing from the spirit and scope of the invention as described in the following claims.

What is claimed is:

1. An analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top insert positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top insert and the porous membrane, wherein the top insert comprises a window positioned over a portion of the porous membrane; and

a bottom insert positioned at a spaced distance below the porous membrane such that a second cavity is formed between the bottom insert and the porous membrane.

2. The device of claim 1, wherein the porous membrane comprises pores having a diameter between about 0.2 microns to about 12 microns.

3. The device of claim 1, further comprising a fluid inlet coupled to the first cavity, wherein the fluid inlet conducts fluids from outside the body into the first cavity during use.

4. The device of claim 1, further comprising a fluid outlet coupled to the second cavity, wherein the fluid outlet conducts fluids from the second cavity out of the body during use.

5. The device of claim 1, further comprising a fluid inlet coupled to the first cavity, wherein the fluid inlet conducts fluids from outside the body into the first cavity during use, and a fluid outlet coupled to the second cavity, wherein the fluid outlet conducts fluids from the second cavity out of the body during use.

6. An analyte detection system comprising:

an analyte detection device, the analyte detection device comprising:

a body;

a porous membrane coupled to the body;

a top insert positioned at a spaced distance above the porous membrane such that a first cavity is formed between the top insert and the porous membrane, wherein the top insert comprises a window positioned over a portion of the porous membrane; and

a bottom insert positioned at a spaced distance below the porous membrane such that a second cavity is formed between the bottom insert and the porous membrane.

a detector optically coupled to the porous membrane, wherein the detector is configured to view at least a portion of the membrane through the window;

a fluid delivery system coupled to the analyte detection device, wherein the fluid delivery system is configured to deliver fluid samples to the analyte detection device.

7. The system of claim 6, wherein the detector comprises a CCD camera.

8. A method of sensing an analyte in a fluid comprising:

passing the fluid across a porous membrane configured to capture the analyte on the porous membrane;

detecting an image of matter captured on the porous membrane; and

determining if the analyte is present on the porous membrane.

9. The method of claim 8, further comprising passing the analyte to a sensor array if the image meets the user-defined criteria.

10. The method of claim 9, wherein the sensor array comprises a porous particle.

11. The method of claim 8, wherein determining if the analyte is present comprises comparing the shape of the matter to user-defined criteria.

12. The method of claim 8, determining if the analyte is present comprises comparing the size of the matter to user-defined criteria.

13. The method of claim 8, determining if the analyte is present comprises comparing the aggregate area of the matter to user-defined criteria.

14. The method of claim 8, determining if the analyte is present comprises comparing the color of the matter to user-defined criteria.

15. The method of claim 8, determining if the analyte is present comprises comparing the fluorescence of the matter to user-defined criteria.

16. The method of claim 8, determining if the analyte is present comprises comparing the fluorescent intensity of the matter to user-defined criteria.

17. The method of claim 8, further comprising applying a stain to the matter captured on the membrane.

18. A particle for detecting an analyte in a fluid comprising a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm.

19. The particle of claim 18, further comprising a receptor coupled to the surface of one of the pores.

20. A method for forming a porous particle, comprising:

forming an emulsion of a polymeric resin in an aqueous solution;

reducing the temperature of the emulsion to produce the porous particle, wherein the porous particle comprises a plurality of pores having a diameter of less than about 1 μm.

21. A porous particle, formed by the method of claim 20.

22. A method for detecting a microbe, comprising:

passing the fluid over a porous particle configured to capture the microbe; and

detecting the microbe with a detector.

23. The method of claim 22, wherein a receptor configured to receive the microbe is coupled to the porous particle.

24. A system for detecting an analyte in a fluid comprising:

a light source;

a sensor array, the sensor array comprising a supporting member comprising at least one cavity formed within the supporting member;

a particle, the particle positioned within the cavity, wherein the particle is configured to produce a signal when the particle interacts with the analyte during use, and wherein the particle comprises a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm; and

a detector, the detector being configured to detect the signal produced by the interaction of the analyte with the particle during use;

wherein the light source and detector are positioned such that light passes from the light source, to the particle, and onto the detector during use.

25. A method of sensing an analyte in a fluid comprising:

passing a fluid over a sensor array, the sensor array comprising at least one particle positioned within a cavity of a supporting member, wherein the particle comprises a receptor coupled to a polymeric resin, wherein the polymeric resin comprises a plurality of pores having a diameter of less than about 1 μm;

monitoring a spectroscopic change of the particle as the fluid is passed over the sensor array, wherein the spectroscopic change is caused by the interaction of the analyte with the particle.

26. A method of sensing an analyte in a fluid comprising:

passing the fluid across a porous membrane configured to capture the analyte on the porous membrane;

applying a visualization agent to the particles on the porous membrane;

detecting an image of matter captured on the porous membrane with a detector at a plurality of wavelengths of light;

detecting an image of matter captured on the porous membrane at a specific wavelength of light, wherein the specific wavelength of light represents light that is not indicative of the presence of the analyte.

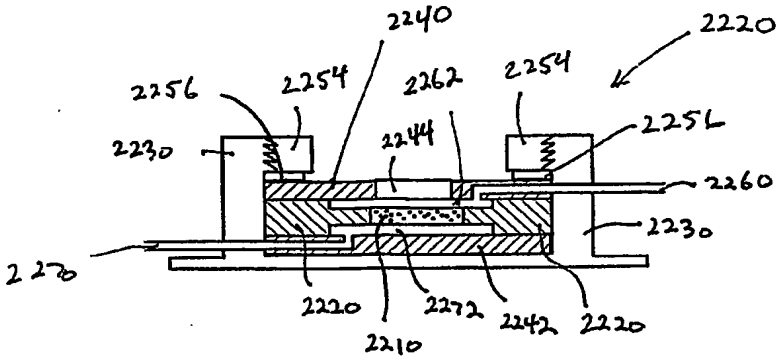

2240
2220
2256
2254
2262
2254
2230
2244
2254
2260
2230
2220
2272
2242
2220
2210

FIG. 1

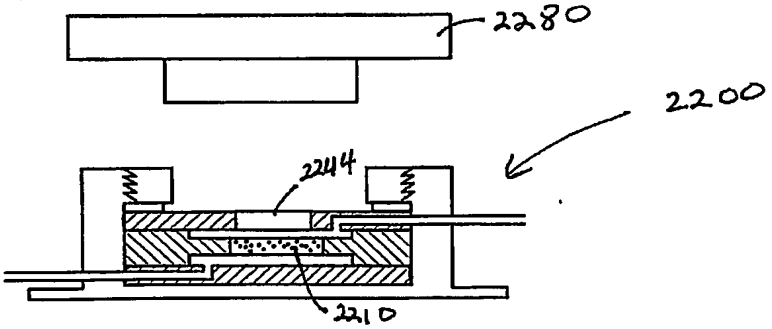

2280
2200
2244
2210

FIG. 2

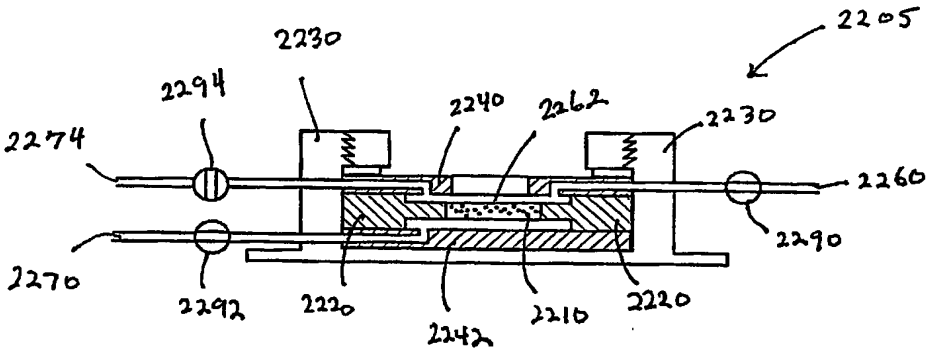

2205
2230
2294
2240
2262
2230
2274
2260
2290
2270
2292
2220
2242
2210
2220

FIG. 3

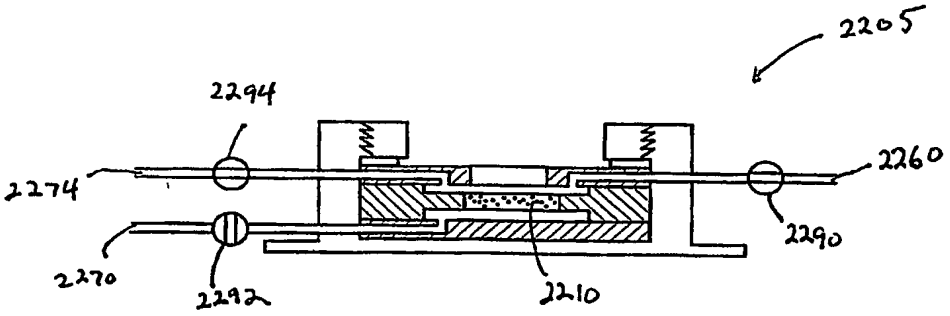

2205
2294
2260
2274
2290
2270
2292
2210

FIG. 4

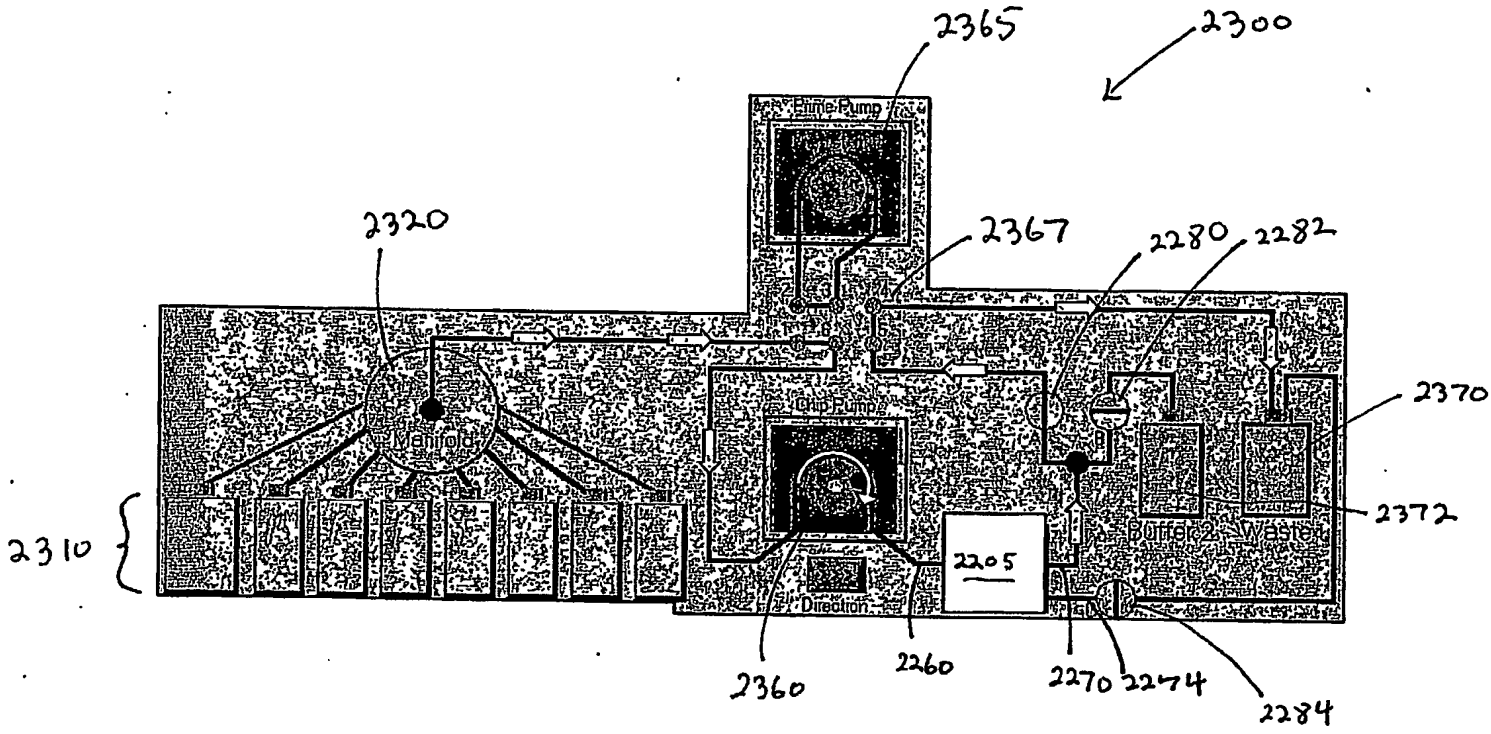

2365
2300
Flume Pump
2320
2367
2280
2282
2370
Manifold
Chip Pump
2372
2310
Buffer 2
Waste
2205
Direction
2360
2260
2270
2274
2284

FIG. 5

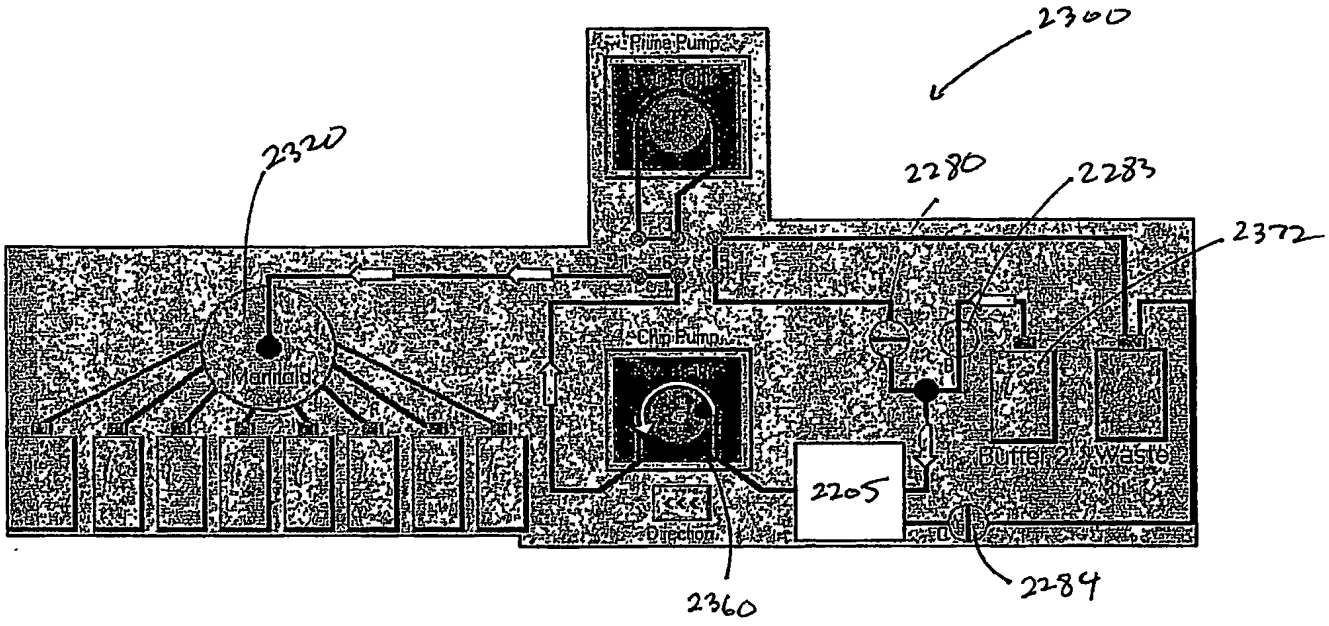

2300
Prime Pump
2320
2280
2283
2372
Chip Pump
Manifold
Buffer 2 / Waste
2205
Direction
2360
2284

FIG. 6

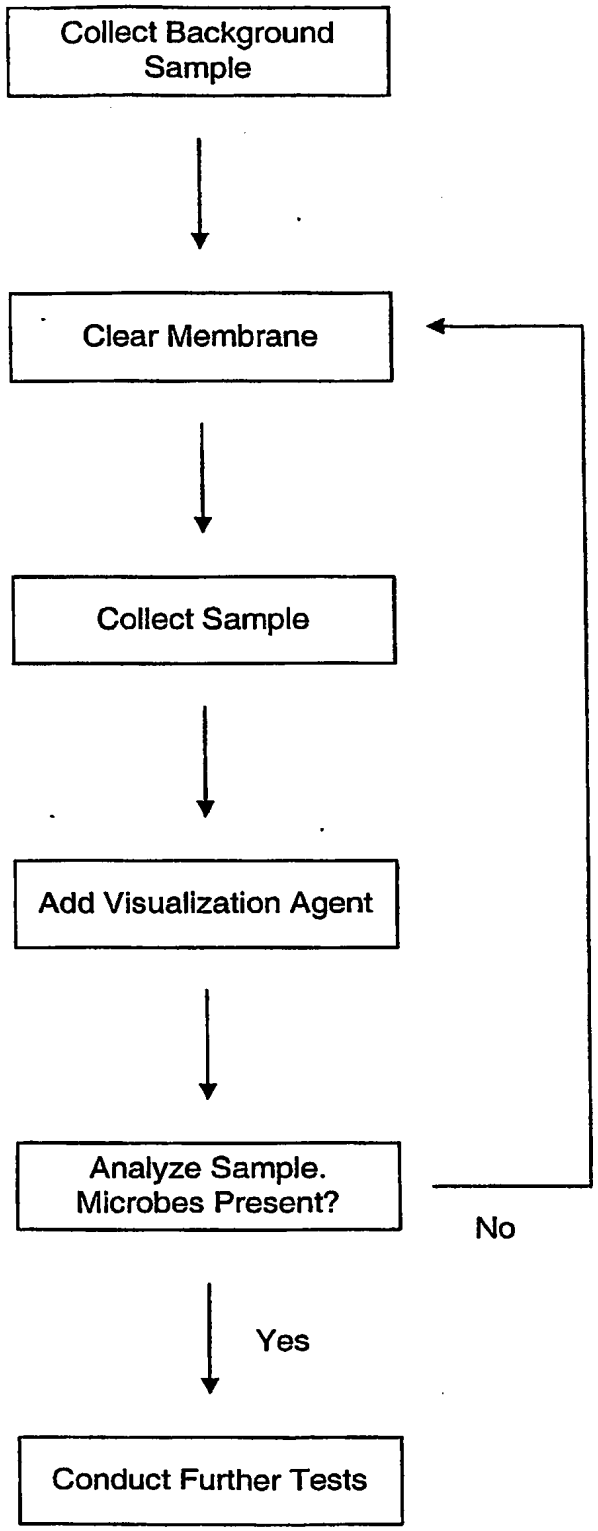

Collect Background Sample
Clear Membrane
Collect Sample
Add Visualization Agent
Analyze Sample. Microbes Present?
No
Yes
Conduct Further Tests

FIG. 7

All Particles

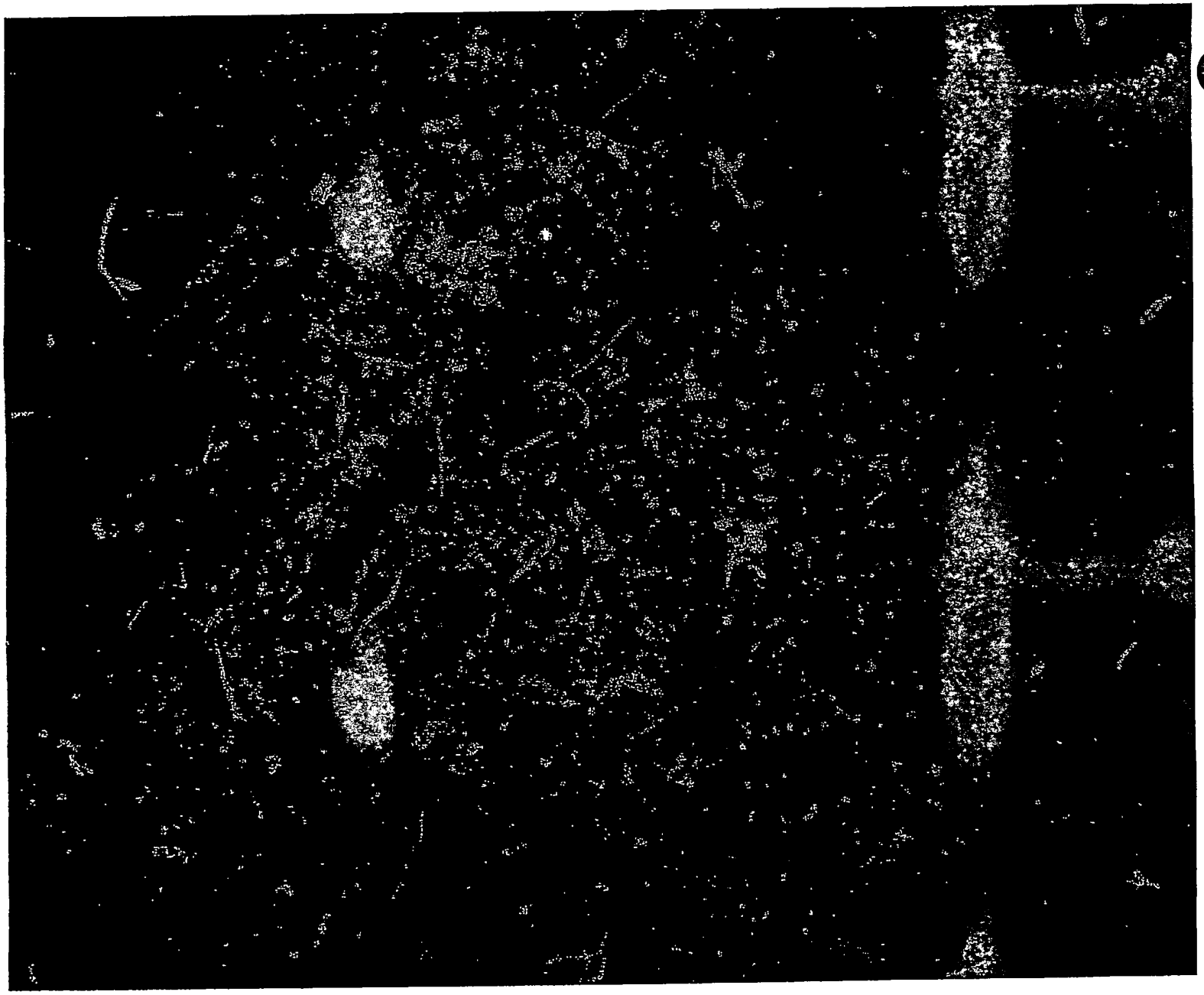


FIG. 8A

Red Mask

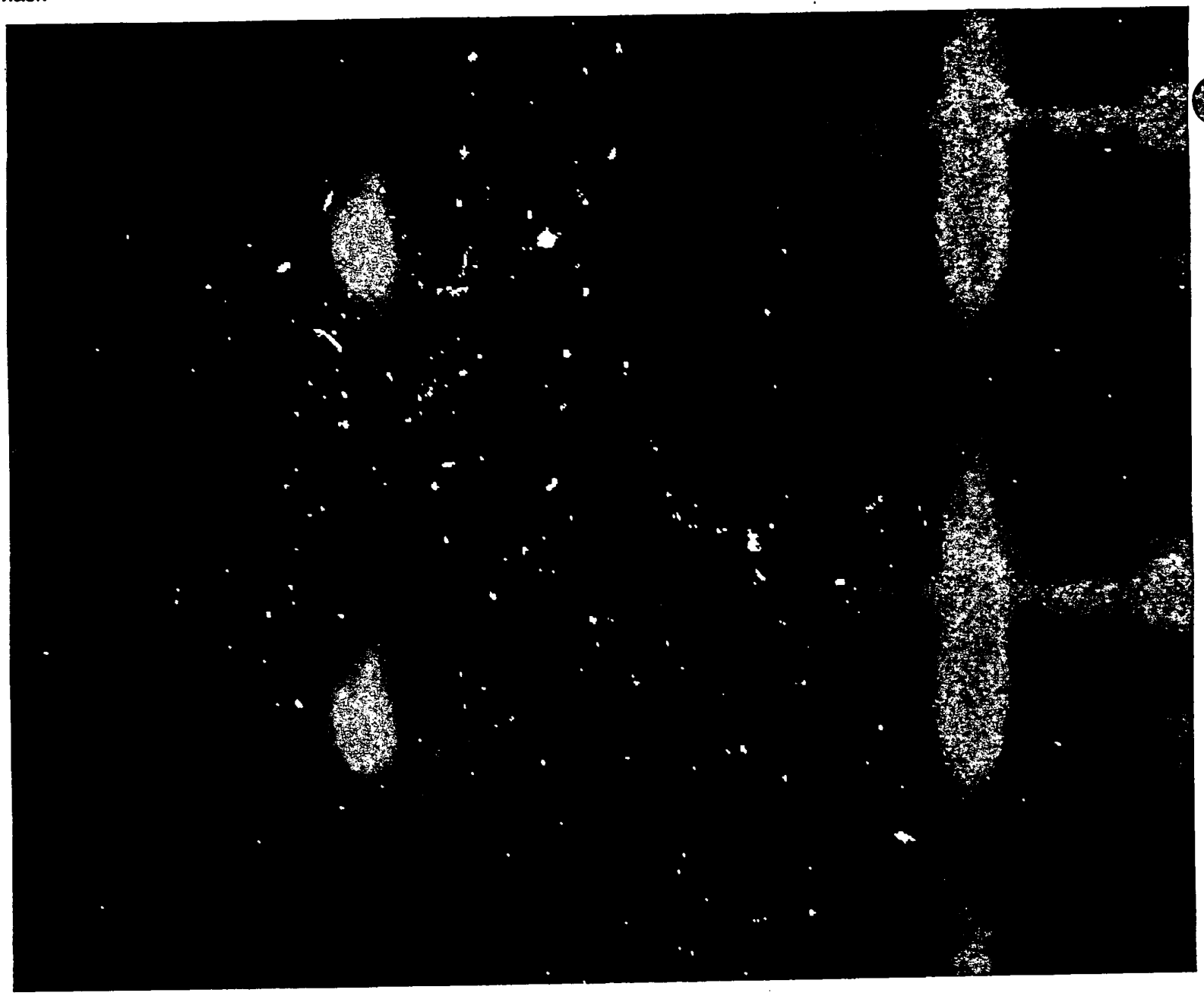


FIG. 8B

All Particles - Red Mask

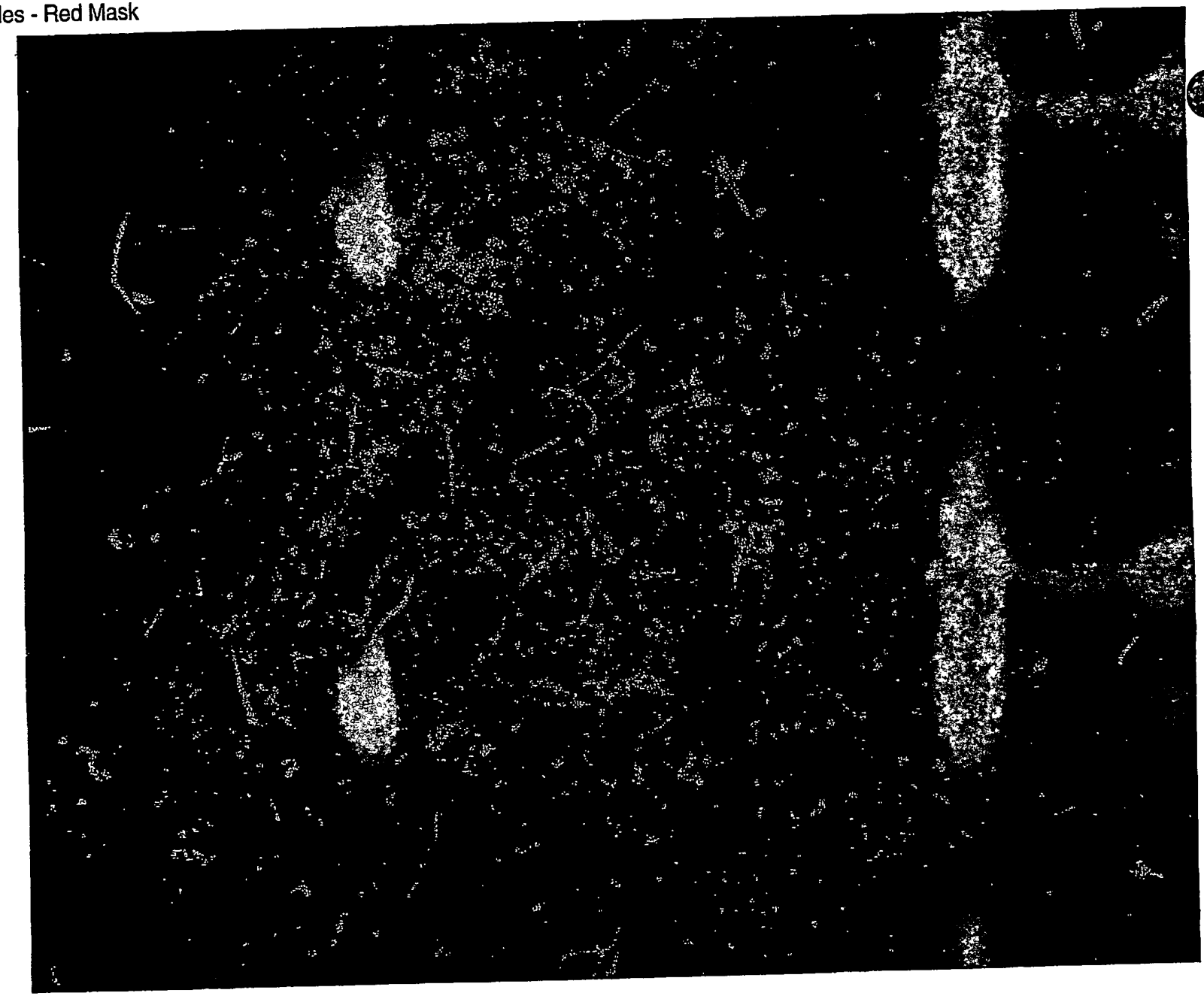


FIG. 8C

20040204.0756

Blue Mask

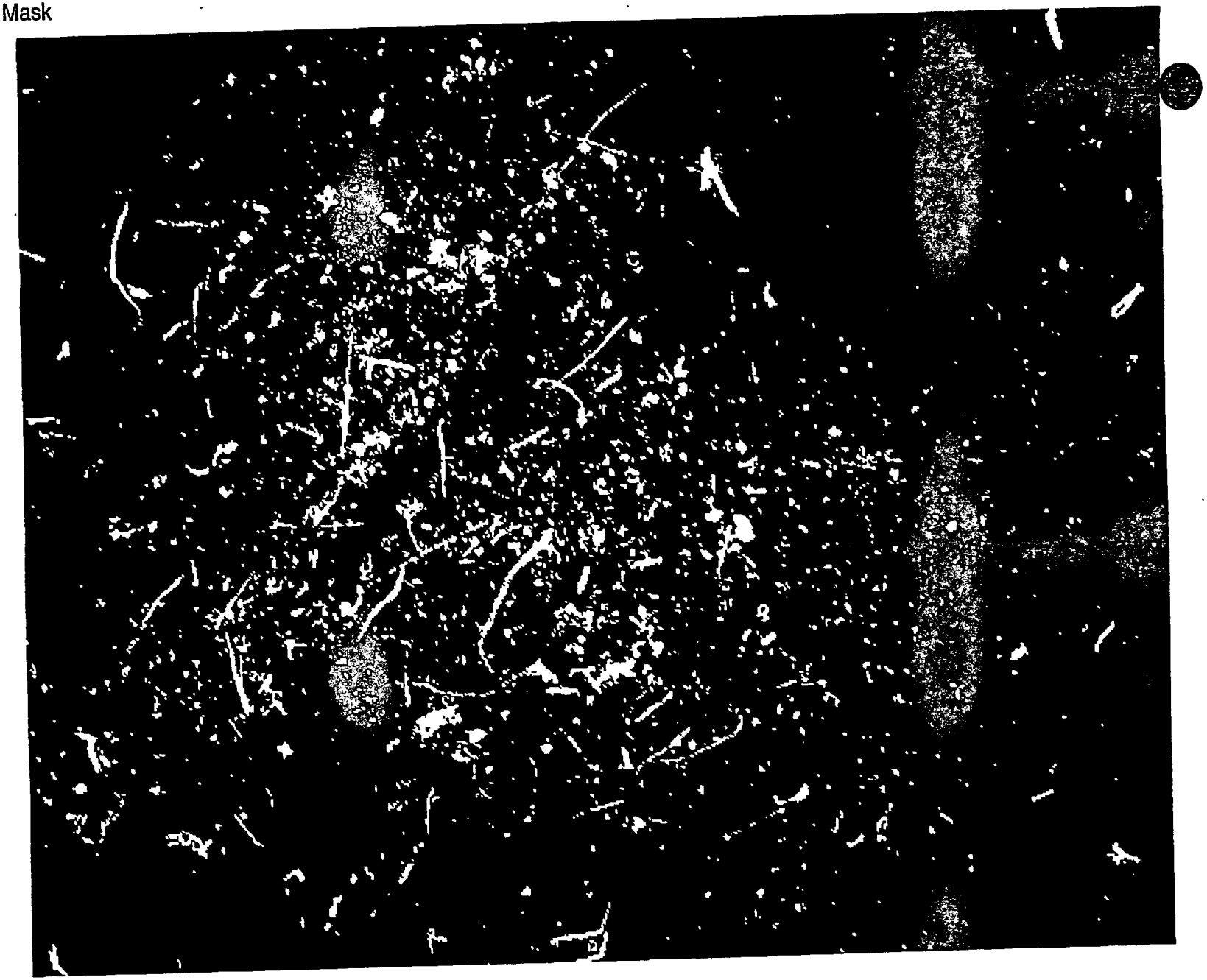


FIG. 8D

All Particles - Red Mask - Blue Mask
(Green Only Particles)

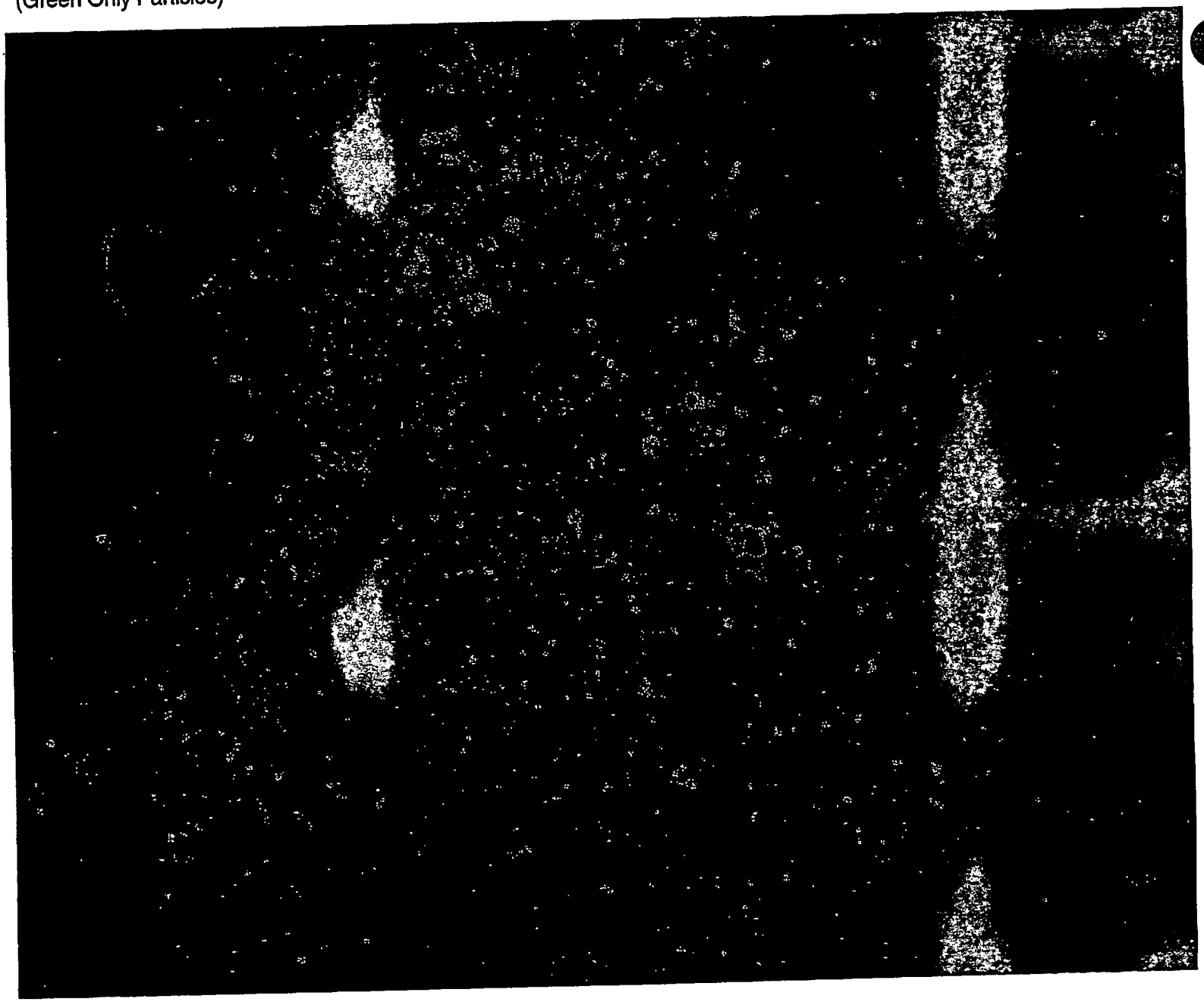


FIG. 8E

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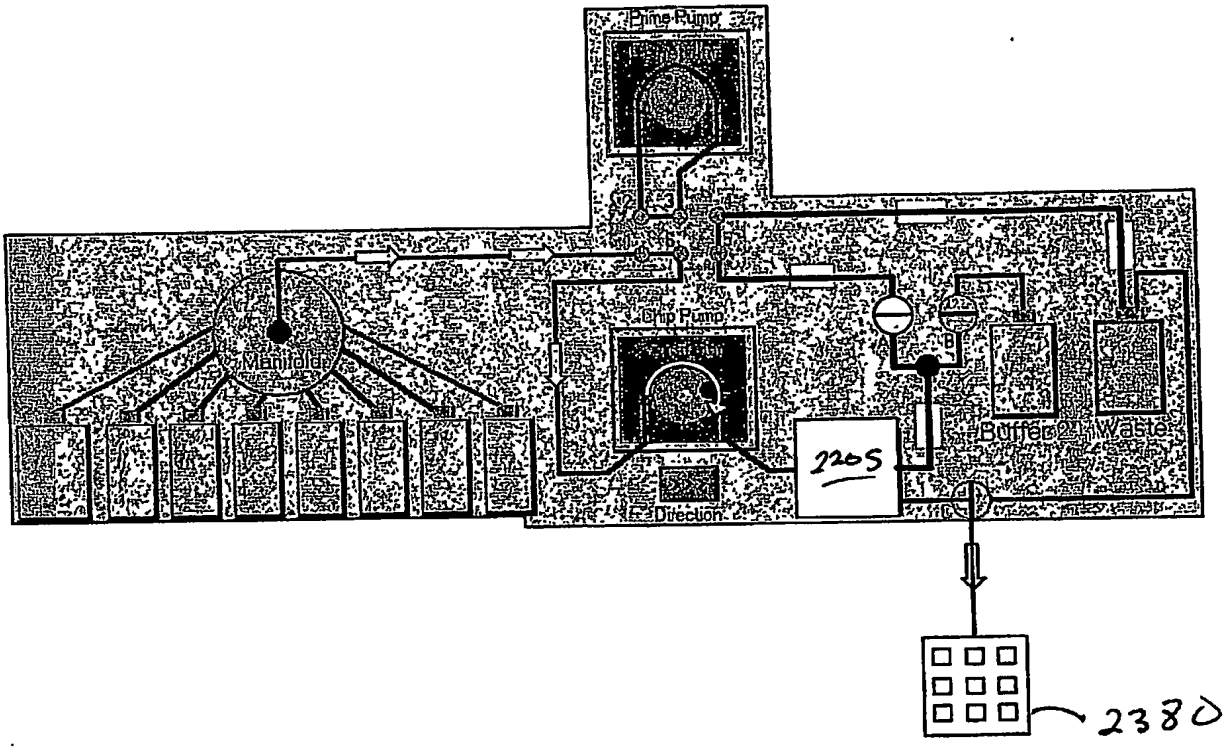

Prime Pump
Drip Pump
Manifold
2205
Buffer 2
Waste
Direction
2380

FIG. 9

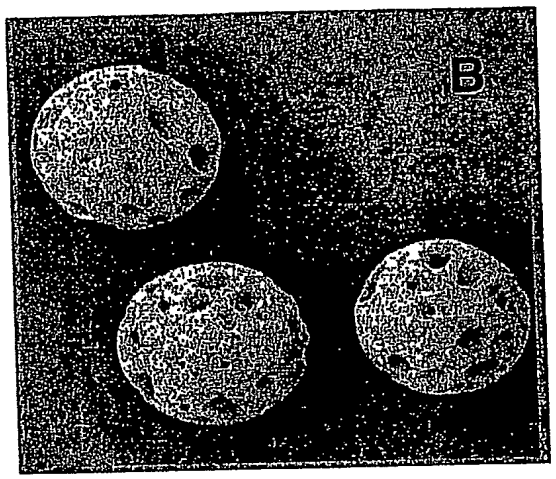


FIG. 10

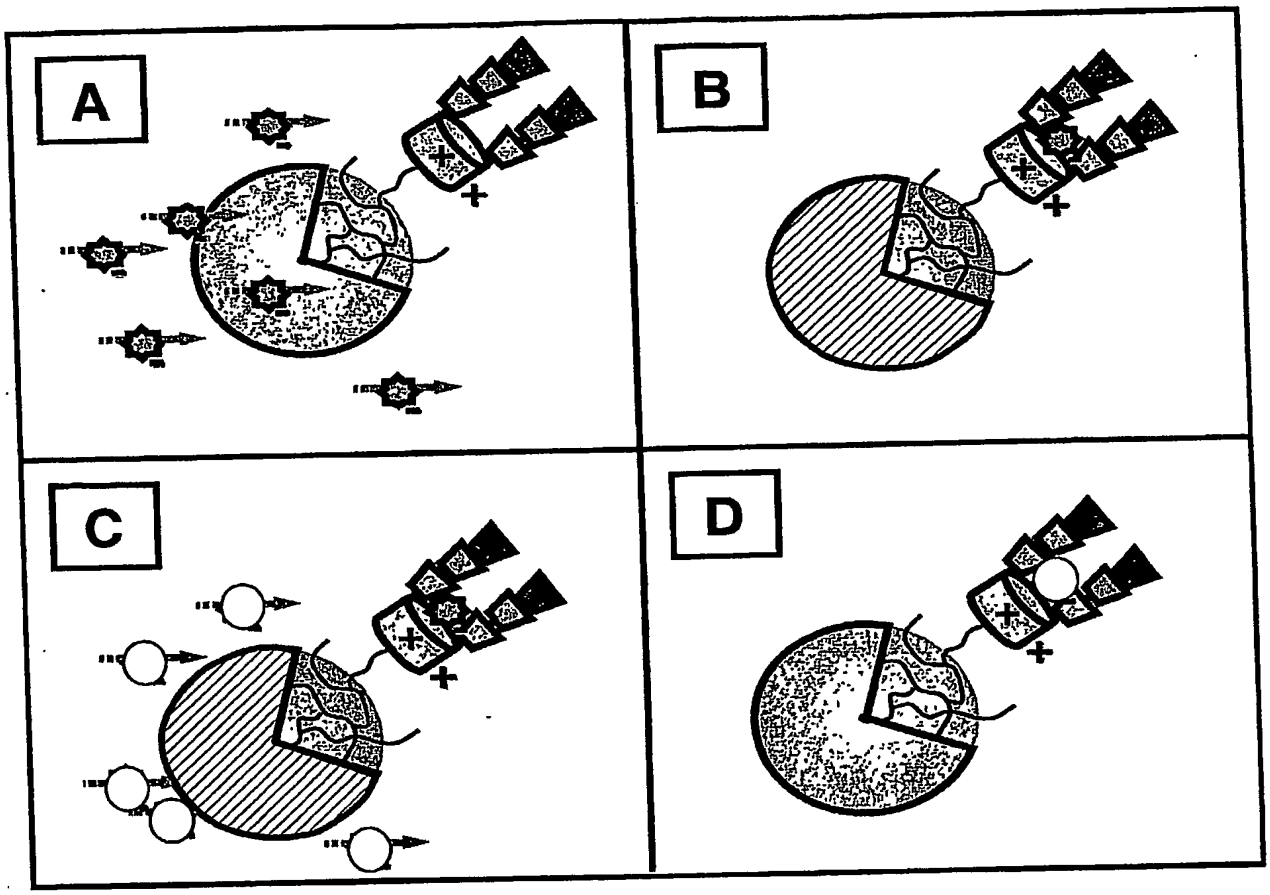


FIG. 11

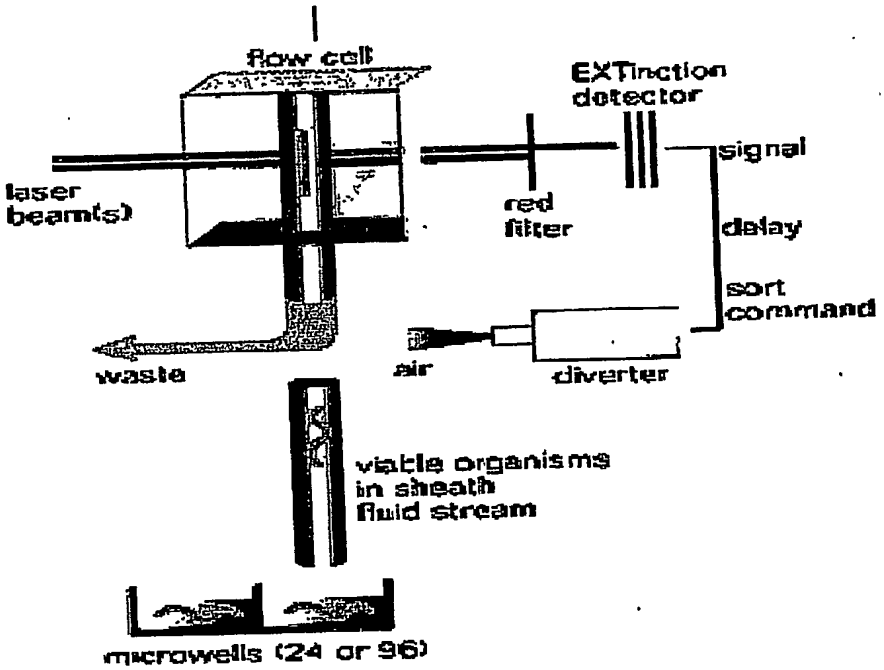

flow cell
EXTinction detector
signal
laser beam(s)
red filter
delay
sort command
waste
air
diverter
viable organisms in sheath fluid stream
microwells (24 or 96)

FIG. 12